

## ACTIVITY RHYTHMS AND BEHAVIORAL CHARACTERIZATION OF TWO EPIGEAN AND ONE CAVERNICOLOUS HARVESTMEN (ARACHNIDA, OPILIONES, GONYLEPTIDAE)

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**ABSTRACT.** The activity rhythms, feeding behavior, and reaction to light of two epigeal (surface inhabitant) species of harvestmen (*Iporangaia pustulosa* and *Iguapeia melanocephala*) and of one cavernicolous species (*Pachylospeleus strinati*) have been recorded. Both the epigeal and the cavernicolous species showed a highly pronounced circadian rhythmicity. The cave species showed a bimodal pattern. Whereas the epigeal species carried food away to feed, the cave species fed where they found the food. The time of reaction to light did not differ statistically between species. However, when exposed to light, the cave species walked much longer distances after it started walking. These differences are probably due to cave adaptation. The cave species may have to wander further for food (and maybe mates) because of the scarcity of resources and, therefore, show greater activity and also a tendency to exploit a resource wherever they find it.

The cave environment is characterized by darkness, at least in deep regions, and the consequent reduction or absence of photoautotrophic organisms, high relative humidity (almost 100%), and small temperature variations over both a daily and annual basis (Barr 1968; Poulson & White 1969; Howarth 1983). These peculiar conditions promote the establishment of a characteristic fauna which may possess morphological, physiological and behavioral modifications that allow them to find food and sexual partners (Culver 1982; Parzefall 1986; Holsinger & Culver 1988), especially among species restricted to the subterranean environment (the troglobites). These modifications may include reduction or loss of eyes and pigmentation, improvement of other sensorial organs, etc. Behavioral patterns that have lost their biological meaning in the new habitat (e.g., avoidance of predators, which may be absent) or for which a cue is absent (e.g., those behaviors related to vision) could be suppressed in the troglobites. In turn, new behavioral patterns related to the new habitat conditions may arise.

One of the most conspicuous biological differences between troglobitic and epigeal species is related to endogenous, self-sustained biological rhythms. Because surface environ-

mental conditions oscillate cyclically, the ability to anticipate temporal changes in the environment would enable an organism to be prepared, both physiologically and behaviorally, to perform specific activities when the environmental conditions are most favorable to the species. This confers to the organism the property of being continuously adjusted to the cyclic changes of the environment and, therefore, of being temporally adapted (Marques & Waterhouse 1994).

In non-troglobitic cavernicolous species (those which may and those which must leave the cave during their life), the presence and synchronization of endogenous rhythms would guarantee the time adjustment of exits and returns to the cave. Indeed, after Saunders (1982), studies focusing on temporal patterns with animals of each one of these categories show that activity patterns appear to reflect the relationship that each animal has with the cave. In contrast, it is generally accepted that the internal clocks of troglobites have been suppressed (Lamprecht & Weber 1991). Nevertheless, as cycles (even attenuated ones) are present in some deep regions of caves, it is possible that some rhythmic characteristics persist.

Although there are numerous troglobitic

species, few studies on biology of opilionids have been conducted (e.g., Juberthie 1964; Gnaspini 1996). We present herein information on activity rhythms and general behavior of three species of harvestmen, one troglotic and two epigean, aiming the comparison of strategies in different habitats and the contribution to the knowledge of biology of opilionids in general.

### METHODS

Three species of Laniatores harvestmen (family Gonyleptidae) were used in this study. The two epigean species, *Iporangaia pustulosa* Mello-Leitão 1935 and *Iguapeia melanocephala* Mello-Leitão 1935, belong to the subfamily Progonyleptoidellinae, and the troglotic species, *Pachylospeleus strinatii* Šilhavý 1974, belongs to the monotypic subfamily Pachylospeleinae. They were chosen for two reasons. First, they are abundant and were easily available for our study. Second, one species is a restricted cave species and the others live only outside of caves. Thus, they have contrasting characteristics.

The species were collected in the Ribeira Valley, São Paulo State, southeastern Brazil. This area is a humid subtropical region without a dry season; total rainfall is 1500 mm; and the annual average temperature ranges between 17–19 °C (Setzer 1966; Monteiro 1973; see also Gnaspini 1996 for description of areas).

Recent phylogenetic analysis of the family Gonyleptidae (R. Pinto-da-Rocha, pers. comm.) has shown that Progonyleptoidellinae is the sister group of Sodreaninae + Caelopyginae. Species of this whole clade (three subfamilies) could be considered “diurnal” because they can easily be seen during the day, active or inactive. On the other hand, Pachylospeleinae belongs to a “nocturnal” clade. Epigean species of this second clade completely hide during the day, leaving their shelters only after dusk.

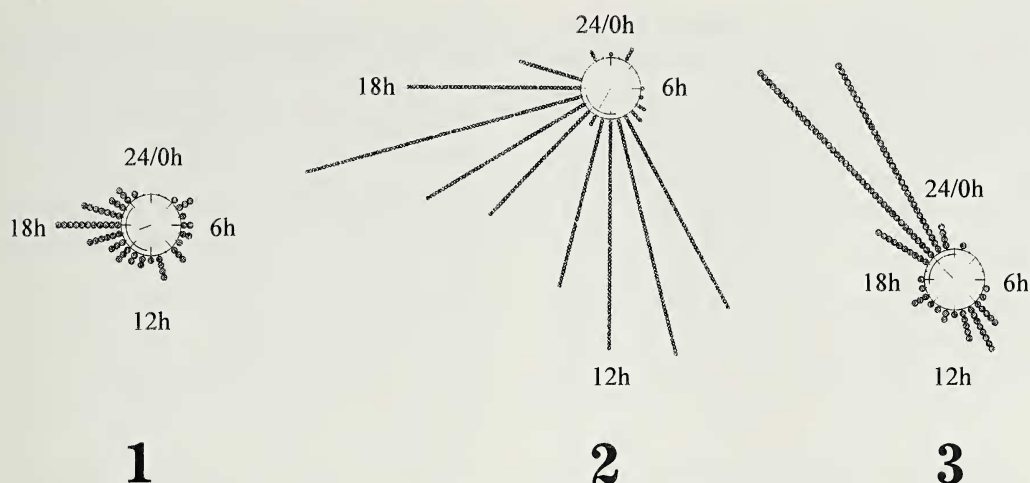
*Iporangaia pustulosa* and *I. melanocephala* are epigean species which live on tree trunks and on or under leaves in rainforests from southeastern Brazil. Our observations have shown that they are not gregarious species because the specimens have been always observed alone or, at most, wandering near conspecifics. Specimens of *I. pustulosa* are generally seen walking on low vegetation near

damp areas during the morning, whereas specimens of *I. melanocephala* are generally seen walking on tree trunks on the same areas. These two species were collected in the area of Parque Estadual Intervales (PEI), a mountainous region with elevation ranging from 70–1000 m (collections were made at about 800 m). Nevertheless, their geographical distribution includes the area where the third species studied is found.

The troglotic species (*P. strinatii*) was collected in the Águas Quentes cave (SP-016, elevation 180 m), located in Parque Estadual Turístico do Alto Ribeira (PETAR), at lower elevations than PEI. It is restricted to one system of caves (“Sistema das Areias”) and has been studied primarily at the population level by Pinto-da-Rocha (1996). It does not seem to be gregarious either. Its endemic distribution, allied with troglomorphisms (depigmentation and increased segmentation of tarsus of the sensorial leg II), have led Pinto-da-Rocha (1996) to consider it a troglote. Šilhavý (1974) has also considered it a troglote, and has added to the list of troglomorphisms the reduction of eyes. However, the species has eyes, which seem to have “normal” size.

We maintained all harvestmen species in terraria with very damp soil on the bottom. Seven individuals of *I. pustulosa* and one of *I. melanocephala* were kept under natural daylight: dark cycles, and four individuals of the cavernicolous *P. strinatii* were kept in a basement laboratory. Inside the laboratory the terraria were left in a chamber with a dark and humid environment which approximates the cave environment. All species were fed once a week with pieces of *Tenebrio obscurus* larvae. *Iporangaia pustulosa* was also fed with pieces of carrot and lettuce. During feeding, we observed behavior under natural illumination or with fluorescent bulbs.

We conducted the tests of reaction to light in a box 35 cm long with a small side retreat in the back. A glass with water provided a thermal barrier between the front of the box and an incandescent bulb (light intensity at this point = 340 lux). An individual was placed in the box 15 minutes before the test, in the end nearest the lamp (which was off) under very dim light (4 lux). During tests, the light was switched on, and the time elapsed for the animal to start walking (time of reaction) as well as the total distance walked dur-



Figures 1–3.—Results of the circular analysis of the activity rhythms of the harvestmen. 1. *Iporangaia pustulosa*; 2. *Iguapeia melanocephala*; 3. *Pachylospeleus strinatii*. The circumference represents 24 hours; each line represents the grouped data (all days recorded) of percentile activity per hour.

ing 15 minutes were recorded. Tests were conducted in different hours of the day, and, in each test, all animals have been tested at the same time. This procedure avoided both reaction differences due to endogenous timing, and differences due to specific different periods of rest/activity. The results were compared using analysis of variance (ANOVA) (Zar 1996).

Continuous records of activity rhythms were made using a system that detects vibrations and sends the data to a computer. Through acoustic sensors connected to the walls, the system recorded overall activity, by detecting all body movements of the animals, including walking, grasping and chewing the food, cleaning legs with mouth parts, etc. All records were made under continuous red light (2 lux, 680 nm), because it is well accepted among entomologists and arachnologists that this type of light does not affect the behavior of these arthropods. Moreover, previous tests showed that this light condition do not disturb the behavior of these animals. The animals were provided with food and water in the beginning of the experiment and after one week. Data were analysed using the Rayleigh test, which tests if there is a preferential direction for a circular unimodal distribution. When Rayleigh was inappropriate, the Hodges-Ajne test, which can be applied to samples with any distribution, even multimodal, was used (Zar 1996).

## RESULTS

**Activity rhythms.**—The results of the records of activity rhythms are presented in Figs. 1–3. The circumference represents 24 h; each line represents the grouped data (all days recorded) of percentile activity per hour. Only the clearest record for each species is shown here.

The record of *I. pustulosa* was made over 7 days. The circular analysis (Fig. 1) indicates that the activity rhythm has a circadian periodicity (Rayleigh test:  $R = 24.18$ ;  $r = 0.41$ ;  $n = 59$ ;  $P < 0.05$ ; this test is more significant the closer  $r$  gets to 1.0), with the major phase of activity occurring around 1700 h. A second and much lower activity peak occurs during the morning.

The record of *I. melanocephala* continued for 11 days. The circular analysis (Fig. 2) also shows a circadian periodicity (Rayleigh test:  $R = 350.43$ ;  $r = 0.693$ ;  $n = 506$ ;  $P < 0.05$ ), with an activity peak around 1400 h.

The record of *P. strinatii* was made for 10 days. The circular analysis (Fig. 3) shows a circadian periodicity in the activity rhythm. However, the activity pattern was clearly bimodal, thus the Rayleigh test could not be applied. In order to statistically analyze these data, we utilized the test of Hodges-Ajne, that indicates a very significant circadian periodicity (Median = 21 h;  $n = 120$ ;  $m = 8$ ;  $P < 0.05$ ). This means a bimodal preference for

Table 1.—Results of the reaction to light of two individuals of *Iporangaia pustulosa* and two of *Pachylospeleus strinatii*. T.R. = Time to start Reaction (min); T.D.W. = Total Distance Walked (cm). “—” represents no displacement.

Date (Time)	<i>I. pustulosa</i>				<i>P. strinatii</i>			
	ind. 1		ind. 2		ind. 1		ind. 2	
	T.R.	T.D.W.	T.R.	T.D.W.	T.R.	T.D.W.	T.R.	T.D.W.
13/Jan/98 (1000h)	4.83	10	5.38	25	2.97	257	7.5	253
16/Jan/98 (1000h)	—	—	4.833	15	12.17	41	—	—
19/Jan/98 (0400h)	1.5	18	1.9	93	0.42	203	17.8	55
22/Jan/98 (1600h)	—	—	0.5	63	1.2	184	—	—
29/Jan/98 (1400h)	—	—	7.65	27	3.6	28	10.4	27

activity at 0900 and 2100 h. The record also shows that this species walks long distances, because the activity is very intense.

**Feeding behavior.**—Neither the epigeal nor the cavernicolous species showed any pattern of intraspecific aggressive behavior, even when feeding occurred after a long time under starvation conditions. Instead, individuals of *I. pustulosa* stayed very close, touching each other with their sensorial legs while feeding. Even interspecific aggressiveness among *I. pustulosa*, *I. melanocephala* and *P. strinatii* was not observed. In turn, in previous observations, individuals of *Goniosoma proximum* (Mello-Leitão 1922) actually expelled *I. melanocephala* from the food (S. Hoenen, pers. obs.). The former are much larger than the latter. *G. proximum* (Goniosomatinae) can be found either in the same area where the two epigeal species occur, either in some caves. The animals studied have been collected from a granitic cave. Whether or not it is a proper cavernicolous species or if it is an accidental in caves is difficult to assure, because they may or may not inhabit caves in areas where they occur in the forest (see Gnaspini 1996 for discussion).

We observed that all epigeal tested species remove pieces of food to ingest away from the source. This movement away from the food source appears to be mediated by contact. The harvestmen seem to stop for ingestion only after they leave the area where they touch one another. This could be a behavior to avoid fighting for food among conspecifics. However, no aggressive pattern was observed when individuals casually meet each other. Moreover, this behavior occurred only when small pieces of food (e.g., pieces of beetles

and of carrot) were offered; when lettuce was offered, possibly because pieces were bigger, they ingested it in the same site, even if touching one another. Although they may rest close to each other, either intra- or interspecifically, they do not seem to be gregarious because resting close together is not the general rule. Because the animals did not exclusively retreat under shelters either before or after food capture, we do not believe that movement away from the food source is related to predator avoidance.

In contrast, this behavior of carrying food did not happen frequently in the troglolithic harvestmen. This could be related to life in an environment lacking predation pressure; the animal does not need to hide while feeding. In addition, it is probably advantageous to immediately consume food when it is patchy and scarce, like in a cave.

**Reaction to light.**—The results of the tests, made with two individuals of *I. pustulosa* and two of *P. strinatii*, are shown in Table 1. In order to evaluate possible differences in these responses, all values of time of reaction obtained for *I. pustulosa* were compared with those obtained for *P. strinatii* using an ANOVA. The same test was used for comparison of the total distance walked. No statistical difference concerning time of reaction was observed between the species (F-ratio = 1.670;  $df = 1$ ;  $P > 0.05$ ). However, there is a statistical difference for the distance walked (F-ratio = 5.514;  $df = 1$ ;  $P = 0.03$ ), indicating that *P. strinatii* walks for significantly greater distances than *I. pustulosa*. This is an interesting result because it suggests a greater vagility in the former species, which is an expected characteristic for a troglolite that lives in an en-

vironment with a poor food supply. Moreover, considering the apparatus used for tests with only 35 cm length, the great distances walked by *P. strinatii* implies that the animal would walk back and forth in the chamber, sometimes towards the light. This may suggest that this species has less photophobic reaction than the epigeal one. However, the fact that the animals react immediately to a light source could imply reaction to a sudden stimulus, and not necessarily a photophobic reaction. We expect that any other stimulus, in addition to light (mechanic, magnetic, electric, etc.), strong enough to be detected by the animal, would promote start of activity. However, we have not tested it yet.

### DISCUSSION

Both of the epigeal species (*I. pustulosa* and *I. melanocephala*) show strong circadian activity rhythms, as expected facing the ubiquity of circadian clocks among surface organisms (Bünning 1967; Menna-Barreto 1997). Both species could also be characterized as "diurnal" because of the main expression of activity during the day. Accordingly, species of the whole clade that includes both *Iporangaia* and *Iguapeia* are considered "diurnal" because they are mostly seen during the day. There are two peaks for *I. pustulosa*, one after dawn and one around sunset, the latter being greater. This pattern may be explained by the temporal distribution of their "food," i.e., insects are much more available during these hours of the day, especially near sunset.

Although it is generally accepted that troglobites have lost temporal organization, at least for circadian frequencies (Lamprecht & Weber 1991), because they live in an environment without the light/dark cycle, some troglobitic species appear to maintain a circadian rhythm (e.g., Wilkens *et al.* 1990; Trajano & Menna-Barreto 1995). This is also the case of *P. strinatii*, which also shows circadian rhythmicity. This seems to be a rather intriguing result, and there are several evolutionary traits that may lead to this result. As pointed out by Husson (1971): "Cave fauna are heterogeneous, differing from one another in the age of their existence, their origins, their reactions towards environment, in the reasons for their presence in caves. On account of this heterogeneity in the cave fauna it is not reasonable to apply identical laws to all cave animals and

to hope to find the same biological rhythms." Therefore, it is not surprising that the temporal aspects of cave species, mainly the troglobites, are not as universal as those of the epigeal species. The main cause for this difference is probably the diversity of ecological origins of troglobites and their adaptive characteristics (Vermeij 1987).

Our data point to an endogenous control underlying the expression of the activity of *P. strinatii*; and, although bursts of locomotion can happen at anytime, there are two main intervals within which activity seems to occur. A circadian rhythm in troglobites may be maintained either because it is advantageous, or because it is a relictual feature from an "old" epigeal relationship which has not been lost. If a given troglobitic species feeds on material ruled by epigeal cycle, it is expected that this species would keep circadian rhythmicity. This would apply, for example, either to a predatory troglobite which feeds on non-troglobitic organisms (which would probably show circadian rhythmicity), or to a detritivorous/scavenger/omnivorous troglobite which feeds on material which comes from the epigeal environment following circadian rhythms (such as regular floods, regular wind flows). However, this does not seem to be the case for *P. strinatii*, as it seems to be mainly detritivorous/omnivorous and the input of organic material in the caves where it lives does not seem to be related to any daily event. On the other hand, the circadian rhythmicity of *P. strinatii* could be considered a relictual feature because Pachylospeleinae (which includes *Pachylospeleus*) belongs to a "nocturnal" clade, in which epigeal species completely hide during the day and leave their shelters only after dusk.

Based on our data, it seems that any given stimulus, be it internal (e.g., hunger), or external (e.g., turning a light on or handling the animal), causes the start of activity of *P. strinatii*, which continues for long time intervals. This happens probably because of the scarcity of food and mates in the environment where the species lives. It is likely that, because food is patchy and scarce and should be exploited promptly, this species feeds wherever it finds food and does not take it away. In addition, the bimodal pattern of activity of *P. strinatii* may be a result of its life in the cave environment; i.e., the scarcity of food may have led

the species to look for food in a more frequent and more intensive way, causing the original nocturnal expression of the activity to become duplicate, resulting in bimodality. However, it awaits testing.

Circadian periodicities seem to be important, not only for adjustment to that habitat, but also for the maintenance of internal temporal organization (Marques *et al.* 1997), which is responsible for the regulation of different and sometimes incompatible physiological systems. Thus, an additional hypothesis is that *P. strinati* could be maintaining a circadian rhythmicity for some internal and yet unknown physiological feature.

### ACKNOWLEDGMENTS

We thank Fundação Florestal do Estado de São Paulo which allowed collections. This study was supported by grant #96/2494-3 from FAPESP. The junior author has a research fellowship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). F.H.S. Santos (IBUSP) is thanked for collecting specimens used for study; and T.C. Ramos (IBUSP) helped in the statistical analysis.

### LITERATURE CITED

- Barr, T.C. 1968. Cave ecology and the evolution of troglobites. *Evol. Biol.*, 2:35–102.
- Bünning, E. 1967. *The Physiological Clock*. The English Universities Press, Springer-Verlag New York, Inc.
- Culver, D.C. 1982. *Cave Life: Evolution and Ecology*. Harvard Univ. Press, Cambridge.
- Gnaspini, P. 1996. Population ecology of *Goniosoma spelaeum*, a cavernicolous harvestman from south-eastern Brazil (Arachnida: Opiliones: Gonyleptidae). *J. Zool.*, 239(3):417–435.
- Holsinger, J.R. & D.C. Culver. 1988. The invertebrate cave fauna of Virginia and a part of eastern Tennessee: Zoogeography and ecology. *Brimleyana*, 14:1–162.
- Howarth, F.G. 1983. Ecology of cave arthropods. *Ann. Rev. Entomol.*, 28:365–389.
- Husson, R. 1971. Rythmes biologiques et vie cavernicole. *Bull. Soc. Zool. France*, 96(3):301–316.
- Juberthie, C. 1964. Recherches sur la biologie des Opilions. *Ann. Spéleol.*, 19:5–238.
- Lamprecht, G. & F. Weber. 1982. A test for the biological significance of circadian clocks: evolutionary regression of the time measuring ability in cavernicolous animals. Pp. 151–178. *In* *Environmental Adaptation and Evolution* (D. Mosakowski & G. Roth, eds.). Gustav Fischer, Stuttgart.
- Lamprecht, G. & F. Weber. 1991. Spontaneous locomotion behavior in cavernicolous animals: the regression of the endogenous circadian system. Pp. 225–262. *In* *The Natural History of Biospeleology* (A.I. Camacho, ed.). Monografias del Museo Nacional de Ciencias Naturales, Madrid.
- Marques, M.D., D. Golombek & C. Moreno. 1997. Adaptação temporal. Pp. 45–84. *In* *Cronobiologia: Princípios e Aplicações* (N. Marques & L. Menna-Barreto, eds.). EDUSP, São Paulo.
- Marques, M.D. & J.M. Waterhouse. 1994. Masking and the evolution of circadian rhythmicity. *Chronobiol. Int.*, 11(3):146–155.
- Menna-Barreto, L. 1997. O tempo na biologia. Pp. 17–21. *In* *Cronobiologia: Princípios e Aplicações* (N. Marques & L. Menna-Barreto, eds.). EDUSP, São Paulo.
- Monteiro, C.A.F. 1973. A dinâmica climática e as chuvas no Estado de São Paulo: Estudo geográfico sob a forma de atlas. Universidade de São Paulo, Instituto de Geografia, São Paulo.
- Parzefall, J. 1986. Behavioural ecology of cave-dwelling fishes. Pp. 433–458. *In* *The Behaviour of Teleost Fishes*. (T.J. Pitcher, ed.). Croom Helm, London & Sydney.
- Pinto-da-Rocha, R. 1996. Biological notes on and population size of *Pachylospeleus strinati* Šilhavý, 1974 in the Gruta das Areias de Cima, Iporanga, south-eastern Brazil (Arachnida, Opiliones, Gonyleptidae). *Bull. British Arachnol. Soc.*, 10(5):189–192.
- Poulson, T.L. & T.C. Jegla. 1969. Circadian rhythms in cave animals. *Proc. IV Int. Congr. Speleol.*, 4–5:193–195.
- Poulson, T.L. & W.B. White. 1969. The cave environment. *Science*, 165:971–981.
- Saunders, D.S. 1982. *Insect Clocks*. Pergamon Press, Oxford.
- Setzer, J. 1966. Atlas climático e ecológico do Estado de São Paulo. Comissão Interestadual da Bacia Paraná-Uruguai, São Paulo.
- Šilhavý, V. 1974. A new subfamily of Gonyleptidae from Brazilian caves, Pachylospeleinae subfam. n. (Opiliones, Gonyleptomorphi). *Rev. Suisse Zool.*, 81(4):893–898.
- Trajano, E. & L. Menna-Barreto. 1995. Locomotor activity pattern of Brazilian cave catfishes under constant darkness (Siluriformes, Pimelodidae). *Biol. Rhythm Res.*, 26(1):341–353.
- Vermeij, G.J. 1987. *Evolution and Escalation*. Princeton Univ. Press, Princeton.
- Wilkens, H., J. Parzefall & A. Ribowski. 1990. Population biology and larvae of the anchialine crab *Munidopsis polymorpha* (Galatheididae) from Lanzarote (Canary Islands). *J. Crust. Biol.*, 10: 667–675.
- Zar, J.H. 1996. *Biostatistical Analysis*. Prentice Hall, New Jersey. Pp. 407–445.

*Manuscript received 29 April 1998, revised 22 January 1999.*